SOME PROPERTIES OF THE LEUCINE-SPECIFIC tRNA's FROM BACILLUS STEAROTHERMOPHILUS

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1. Introduction

The use of systems extracted from thermophilic organisms e.g. *Bacillus stearothermophilus* make it possible to study the effect of heat on the aminoacylation of tRNA at elevated temperature [1]. The purification and some properties of the leucyl-tRNA synthetase (L-leucine-tRNA ligase (AMP) EC 6.1.1.4) from this bacterium have been described previously [2]. This enzyme was found to be highly thermostable although its complex with the leucyladenylate was just as heat labile as that formed with the corresponding enzyme from *E. coli* [3].

On the other hand, it has been assumed [1,4] that tRNA's extracted from thermophilic bacteria and *E. coli* possess similar properties although no systematic physical studies have been done on this material. In this paper we are presenting some properties of the purified $tRNA_{leu}$'s from *B. stearothermophilus*. We found that *B. stearothermophilus* tRNA contains several isoaccepting species specific for leucine. The thermal denaturation curves of these tRNA's were found to be monophasic and their T_m was considerably higher than that of crude *B. stearothermophilus* tRNA.

Hyperchromicity at 258 and 280 nm is comparable to that found for other specific tRNA's [5].

2. Methods

Organisms, chemicals, purification of aminoacyl-tRNA synthetases and the preparation of unfractionated tRNA from *B. stearothermophilus* have been described previously [2,3]. *E. coli* B tRNA was purchased from Schwarz BioResearch. Hydroxylapatite (Biogel HTP) was a Calbiochem product.

Highly purified *B. stearothermophilus* $tRNA_{leu}$'s used in this study were prepared by reaction of the phenoxyacetyl ester of *N*-hydroxysuccinimide with leucyl-tRNA and stepwise elution on BND-cellulose as described by Gillam et al. [6]. Leucine specific tRNA's from *B. stearothermophilus* were at least 80% pure as judged by their ^{14}C-leucine accepting capacity (1296 pmole/A_{260}).

A 50% pure leucine specific tRNA's fraction from *E. coli* B obtained by the same method was also used in heat denaturation experiments. Analytical chromatography of charged tRNA's on BND-cellulose and hydroxylapatite was performed as described by Gillam et al. (1967) [7] and by Muench and Berg (1966) [8] respectively. The chromatographic separations were run at room temperature and the buffers contained 0.2% NaN_3 to prevent infection.

Heat denaturation experiments were performed in a temperature-controlled Beckman-Gilford or Cary 15 spectrophotometer in 0.01 M tris HCl buffer pH 7.4 as measured at 25°C with or without added 6×10^{-3} M $MgCl_2$. The solutions were degassed before the experiments and stoppered cuvettes were used.

Charging of leucine on tRNA was performed in a standard reaction mixture containing per ml: Sodium cacodylate buffer pH 7.4, 100 μmole; ATP disodium salt, 1 μmole; $MgCl_2$, 6 μmole; reduced glutathione, 4 μmole; ^{14}C-leucine (311 mCi/mmole), 0.05 μCi (for preliminary semi-micro scale purifications and analytical chromatography) or ^{12}C-leucine, 1 μmole and tRNA, 12 A_{260} units.

The amount of enzyme added was such that a maximal quantity of leucine was loaded on tRNA

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after 15 min at 37°C.

In preparative experiments, we usually handled 2400 A_{260} units of crude tRNA at one time and volumes were scaled up proportionally to the quantities of tRNA used.

The tRNA's were obtained from the reaction mixtures by phenol extraction and ethanol precipitation at -15°C.

The yield of the purification procedure was about 12 A_{260} units of specific tRNA's from 2400 A_{260} units of crude tRNA.

3. Results and discussion

3.1. *Multiplicity*

Fig. 1 represents the elution pattern of crude *B. stearothermophilus* ^{14}C-leucyl-tRNA on a column of BND-cellulose (1.5 X 75 cm). The tRNA (48 A_{260} units of ^{14}C-leucyl-tRNA and 240 A_{260} units of non-acylated tRNA) was eluted by a linear gradient of NaCl 0.45 to 1 M in 0.01 M sodium acetate buffer pH 4.5 containing 0.01 M $MgSO_4$. The mixing chamber and the reservoir each contained 5 l buffer. The flow rate was maintained at 60 ml/hr by means of a peristaltic pump; fractions of 15 ml were taken, their A_{260} measured and their radioactivity determined in a Packard Tricarb Scintillation spectrometer using a toleune-triton medium containing PPO and POPOP.

It can be seen that the tRNA's esterified with leucine elute as two very well separated peaks (peaks I and II).

Each peak was collected and concentrated by means of a Diaflo pressure dialysis set with a UM 10 membrane and tRNA was precipitated at −15°C by cold absolute ethanol.

In the case of the peak II,48 A_{260} units of crude *B. stearothermophilus* tRNA were added as carrier.

Peaks I and II were further chromatographed on hydroxylapatite using columns of 2.5 X 15 cm and

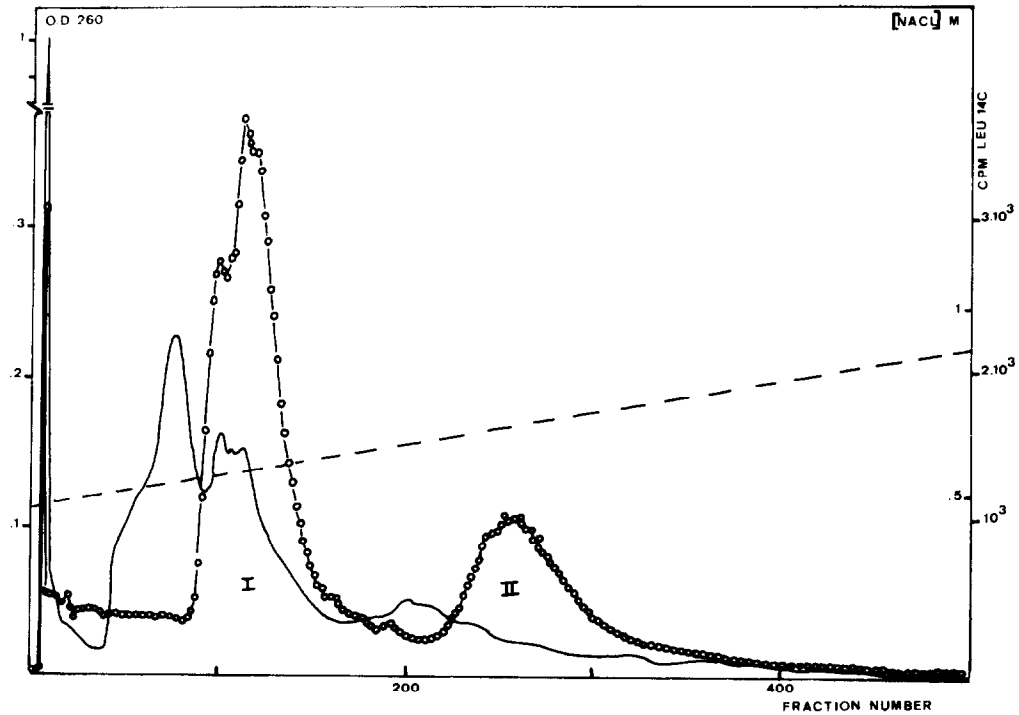

Fig. 1. Chromatography of crude ^{14}C-leucyl-tRNA from *B. stearothermophilus* on BND-cellulose. o—o Radioactivity in CPM/2 ml of fraction. —— Optical density at 260 nm. The gradient is indicated by a dashed line. For experimental details see text.

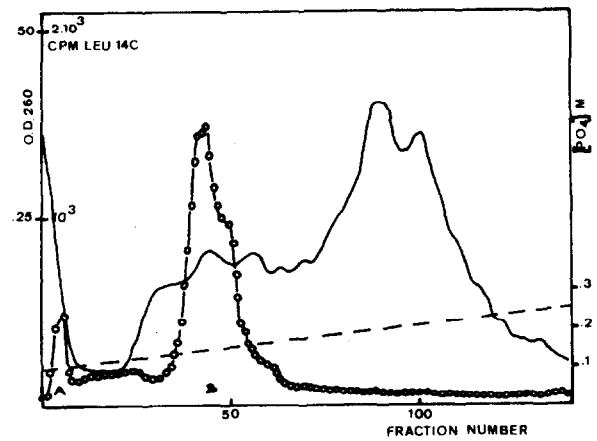

Fig. 2. Chromatography on hydroxylapatite of peak I (see fig. 1). o—o Radioactivity in CPM/2 ml of fraction. —— Optical density at 260 nm. The gradient is indicated by a dashed line. For details see text.

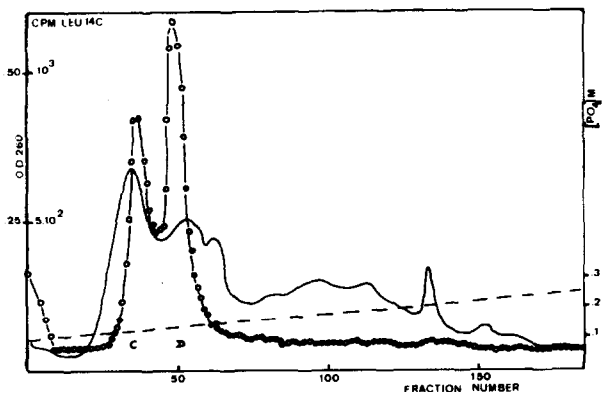

Fig. 3. Chromatography on hydrocylapatite of peak II (see fig. 1). o—o Radioactivity in CPM/2 ml of fraction. —— Optical density at 260 nm (note that unfractionated *B. stearothermophilus* was added as carrier). The gradient is indicated by a dashed line. For details see text.

eluting the tRNA's by a linear gradient of 0.1 M to 0.25 M potassium phosphate buffer pH 5.8 with a total volume of 2 l. The flow rate was 75 ml/hr and fractions of 12.5 ml (peak I) or 10 ml (peak II) were taken.

Fig. 2 shows that peak I is resolved in at least two components (peaks A and B). Peak A has not been rechromatographed, it is not certain whether it represents a separate component. Peak B probably contains more than one species of leucyl-tRNA as can be seen from the shoulder presented by this peak.

Fig. 3 indicates that peak II is clearly resolvable in two species, peaks C and D.

It may be concluded that as in many other organs and organisms [9] there are several isoaccepting tRNA's for leucine in *B. stearothermophilus*. Whether these different species are interconverted forms of tRNA [10,11] is highly improbable since a heat treatment of these tRNA's at 50°C in the presence of 0.02 M $MgCl_2$ or 0.004 M EDTA does not alter their accepting capacity for leucine.

3.2. *Thermal denaturations*

The effect of temperature on the ultraviolet absorption of tRNA was also investigated. The fraction of $tRNA_{leu}$'s from *B. stearothermophilus* used in this study contains all the tRNA's specific for that amino-acid as these are not separated during the purification procedure [6]. For that reason, the results presented here give average values for T_m and hyperchromicity.

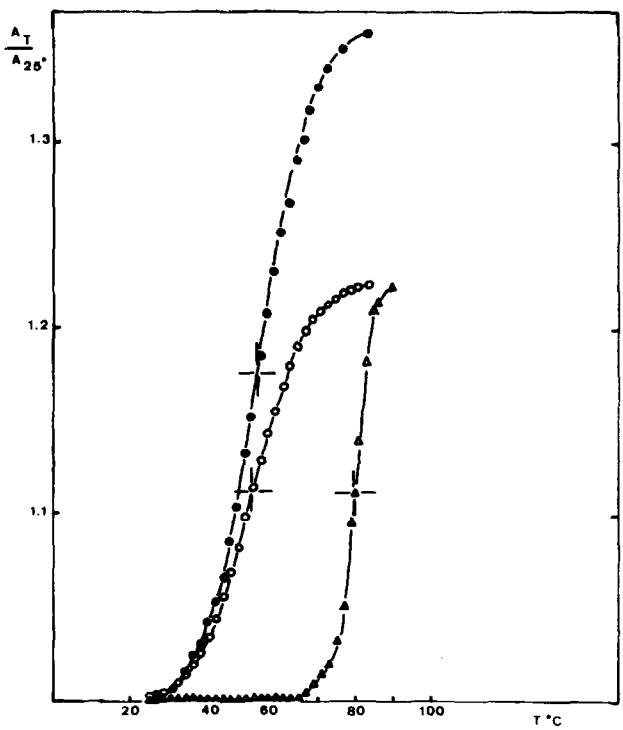

Fig. 4. Melting curves of unfractioned *B. stearothermophilus* tRNA. o—o at 258 nm without added Mg^{2+} ions. Δ—Δ at 258 nm in 6.10^{-3} M $MgCl_2$. •—• at 280 nm without added Mg^{2+} ions.

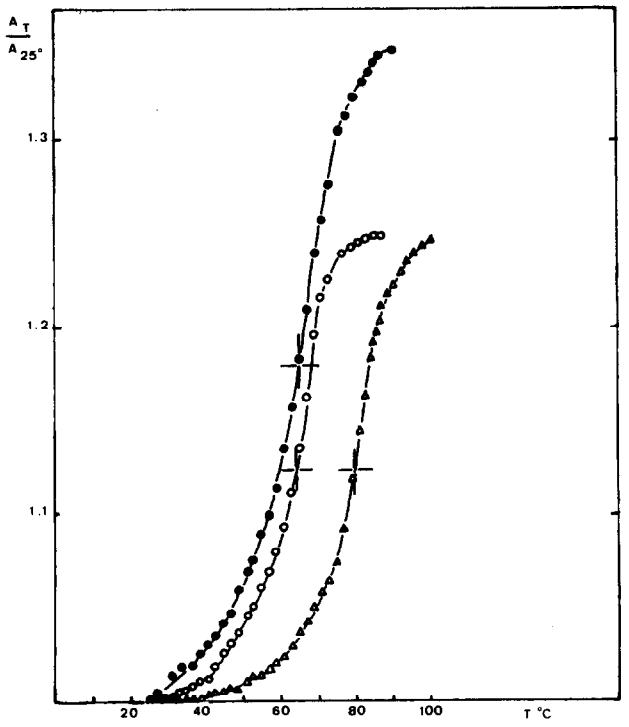

Fig. 5. Melting curves of leucine-specific tRNA's from *B. stearothermophilus*. Symbols as in fig. 4.

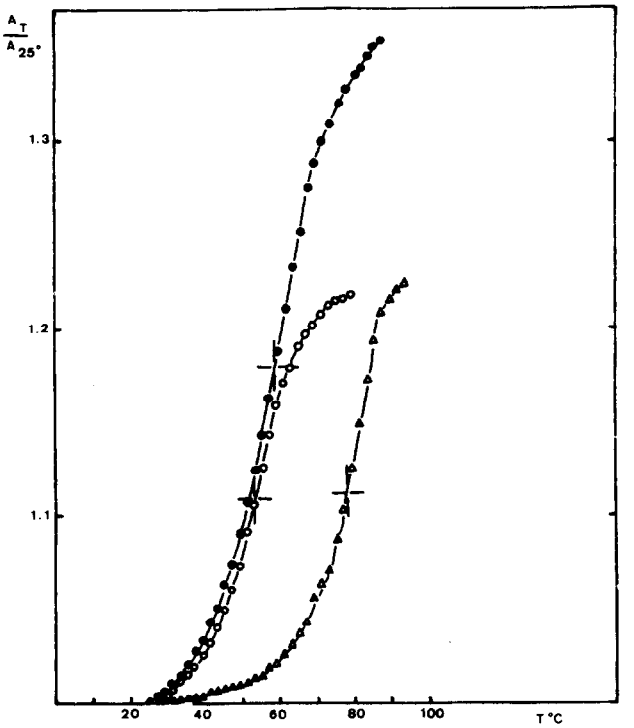

Fig. 7. Melting curves of a 50% pure leucine-specific tRNA's fraction from *E. coli*. Symbols as in fig. 4.

Figs. 4 and 5 represent the melting curves of unfractionated *B. stearothermophilus* tRNA and leucine-specific tRNA's respectively. The curves were recorded at 258 and 280 nm with or without added 6×10^{-3} M $MgCl_2$. As can be seen from these figures, $tRNA_{leu}$'s possess a much higher T_m at 258 and 280 nm (T_m =

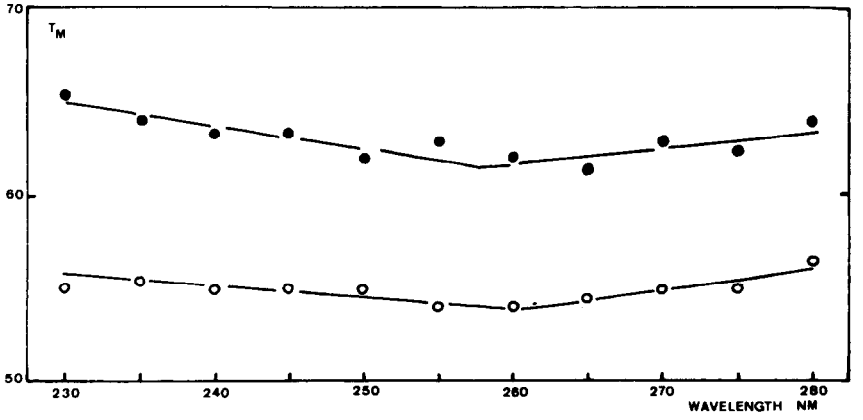

Fig. 6. T_m variation with wavelength in the absence of added Mg^{2+} ions. o—o Unfractionated *B. stearothermophilus* tRNA. •—• Leucine-specific tRNA's from *B. stearothermophilus*.

Table 1
Values of T_m and hyperchromicity of the different tRNA's studied.

	B. stearothermophilus $tRNA_{leu}$'s	*B. stearothermophilus* unfractionated tRNA	*E. coli* tRNA enriched in $tRNA_{leu}$'s
T_m at 258 nm with Mg^{++}	80°	80°	78°
T_m at 258 nm without added Mg^{++}	64°5	53°	52°5
T_m at 280 nm without added Mg^{++}	65°	55°	59°
ΔA_{280} in %	35	35	35
ΔA_{258} in %	25	23.5	20.5

64.5°C and 65°C) than does the unfractionated tRNA (T_m = 53°C and 55°C). On the other hand, their T_m at 258 nm in the presence of added Mg^{2+} ions are identical (T_m = 80° C).

A striking fact is also that their T_m at 258 and 280 nm are very close although the increase in absorbance is much higher at 280 nm than at 258 nm as it is the case for other tRNA's [5]. Indeed, as can be seen from fig. 6, the T_m values for leucine-specific and unfractionated tRNA's from *B. stearothermophilus* present little dependence on wavelength. This is in contrast with the thermal denaturation of a 50% pure $tRNA_{leu}$ fraction from *E. coli* where the T_m at 258 and 280 nm without added Mg^{2+} ions are respectively 52°C (fig. 7). These data are summarized in table 1.

Our results indicate that the leucine-specific tRNA's from *B. stearothermophilus* melt entirely cooperatively since there is no discontinuity in their denaturation curve and since their T_m is almost independent of wavelength [12]. This is also the case of the $tRNA_{phe}$ from yeast [13] whereas other tRNA's exhibit biphasic denaturation [13,14]. The increase in absorbance at 280 nm indicates that unfractionated tRNA and leucine-specific tRNA's from *B. stearothermophilus* contain the same amount of G-C pairs involved in helical regions although the T_m of the latter tRNA's is about 10° higher in the absence of added Mg^{2+} ions. This striking difference in T_m could therefore be due to some heat-resistant tertiary structure stabilizing the $tRNA_{leu}$'s or to some length effect caused by a secondary structure containing long uninterrupted helical base-paired regions as pointed out by Kallenbach [15].

Unusual abbreviations: A_{260} = absorbance at 260 nm; BND-cellulose = benzoylated and naphtoylated diethylamioethyl cellulose; PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis- (phenyloxazolyl-2) benzene.

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